Reference: *Biol. Bull.* **166**: 156–166. (February, 1984)

INACTIVATION OF CELL MOVEMENT FOLLOWING SEXUAL CELL RECOGNITION IN *PARAMECIUM CAUDATUM**

AKIO KITAMURA AND KOICHI HIWATASHI

Biological Institute, Tohoku University, Aoba-yama, Sendai 980, Japan

ABSTRACT

A positive correlation between sexual cell recognition and decrease in swimming velocity was studied in the ciliated protozoan, *Paramecium caudatum*. Ciliary membrane vesicles with conjugation-inducing activity rapidly decreased swimming velocity of living cells of the opposite mating type. Immobilization of the ventral cilia and perturbation of metachronal waves in the dorsal cilia were observed upon application of the membrane vesicles. Anterior shift of the beating direction and decrease in the beating angle or amplitude were also observed in the cells reacting to the vesicles. A similar change of swimming behavior and ciliary inactivation was seen in the chemical induction of conjugation. A possible regulatory mechanism of ciliary movement is discussed.

INTRODUCTION

In many ciliates, cilia, the locomotive organelles, also play an important role as a mating organelle. Thus, conjugation in *Paramecium* is initiated by ciliary agglutination between cells of complementary mating types referred to as the mating reaction (Sonneborn, 1937). Cells in the agglutinative mating reaction adhere to each other at the surface of ventrally located cilia (mating reactive cilia) and appear to move slowly or stop swimming for about one hour until conjugating pairs appear. However, the agglutination of the cells hinders detailed observation of the change in ciliary movement of individual cells. Kitamura and Hiwatashi (1980) reported that ciliary membrane vesicles, prepared by treatment of mating reactive detached cilia with lithium diiodosalicylate, have high conjugation-inducing activity but lack the agglutination-inducing activity. The reaction between the membrane vesicles and cells of the opposite mating type provides a good system for detailed observation of ciliary movement during the mating reaction because the reaction involves the same mechanism as the mating reaction of living cells in the induction of conjugation.

Marked change in swimming behavior upon transferring mating reactive cells into conjugation-inducing chemicals has been reported by many investigators (Miyake, 1958; Tsukii and Hiwatashi, 1978; Cronkite, 1979). However, little is known about ciliary movement of the cells in the conjugation-inducing media and also about the precise relationship between the swimming behavior of cells in the mating reaction and its implication in the mechanisms triggering the succeeding mating processes. Here we report detailed observations of ciliary movement following sexual interaction with mating reactive membrane vesicles and treatment with conjugation-inducing chemicals, and also discuss possible mechanisms of the inactivation of cell movement.

Received 14 November 1983; accepted 22 November 1983.

* This paper is dedicated to Dr. Mamoru Kusa on his retirement.

Abbreviations: LIS = lithium diiodosalicylate, Tris = tris(hydroxymethyl)aminomethane, MV = membrane vesicles, EDTA = ethylenediamine tetraacetic acid, ATP = adenosine triphosphate, EGTA = ethyleneglycol bis(β-aminoethyl ether)-N,N′-tetraacetic acid.

Materials and Methods

Stocks and culture method

The stocks used were Kyky 1, Ksy-S7a, and 27aG3-S1 of mating type V, and Yt3-G9, d-8b, and 27aG3 of VI in syngen 3 of *Paramecium caudatum*. Sexually immature cells were obtained from selfing progeny of 27aG3-S1. They are genetically identical to their parent cells because 27aG3-S1 is a complete homozygous clone (Tsukii and Hiwatashi, 1979).

Cells were cultured at 25°C in 1.25% (w/v) lettuce juice medium in Dryl's solution (Dryl, 1959), and inoculated with *Klebsiella pneumoniae* one day before use (Hiwatashi, 1968). Subculture was made frequently to maintain active growth. Stationary phase cells for experiments were obtained by inoculating several hundred cells into 2 ml culture medium, adding fresh medium of 4 ml, 10 ml, and 10 ml on successive days, thus allowing growth to occur to about 2×10^3 cells/ml. At the stationary phase, most sexually mature cells exhibited strong mating activity; immature cells showed no activity. Ca-poor culture medium was prepared to grow cells for chemical induction of conjugation.

Isolation of LIS-membrane vesicles

Ciliary membrane vesicles which can induce conjugating pairs without prior occurrence of mating agglutination were isolated by the method of Kitamura and Hiwatashi (1980). Mating reactive cilia detached by the modified method of Fukushi and Hiwatashi's (Kitamura and Hiwatashi, 1978) were treated with 4 m*M* lithium diiodosalicylate (LIS, Eastman, No 11187) in 10 m*M* Tris-HCl buffer, pH 7.6, for 30 min at 4°C. The concentration of the total ciliary protein in this suspension was about 0.5 mg/ml. The treated cilia were precipitated by centrifugation at $9000 \times g$ for 10 min and the supernate was passed through 0.2 μm Membran-filter (Sartorius SM 11407) to remove possible contamination of remaining cilia fragments. The filtrate was dialyzed against 3 liter of 10 m*M* Tris-HCl, pH 7.3, overnight with a change of the same buffer. The dialysate was centrifuged at $105{,}000 \times g$ for 60 min. The pellet obtained by this procedure always consists chiefly of membrane vesicles which are called the LIS membrane vesicles.

Measurement of swimming velocity

For photographs of swimming behavior, one ml of cell suspensions of 1000/ml to 2000/ml in cell density were applied to glass plates under which water from a temperature-controlled bath was circulating through a thermal stage of glass. Swimming velocity was measured by photographs taken with 2 seconds exposure or stroboscopic pulse exposures. Before measurements, cells were adapted to the solution containing 1 m*M* KCl, 1 m*M* $CaCl_2$ and 1 m*M* Tris-HCl, pH 7.1, for 20 min or longer at 21°C.

Chemical induction of conjugation

Mating reactive cells of a single mating type cultured in the Ca-poor medium were washed twice with and suspended in a solution containing 1 m*M* KCl, 0.03 m*M* $CaCl_2$, and 1 m*M* Tris-HCl, pH 7.1. The cell suspension was mixed in test tubes with the same volume of the inducing medium which contains 19 m*M* KCl, 0.03 m*M* $CaCl_2$, and 1 m*M* Tris-HCl, pH 7.1, at 25°C to give final concentrations of 10 m*M* KCl and 0.03 m*M* $CaCl_2$. The cell density after mixing with the inducing medium was about 2000/ml.

Results

Mating type specific inhibition of swimming by the LIS membrane vesicles

The LIS membrane vesicles (LIS-MV) with high conjugation-inducing activity were prepared from about 100 l culture of stock Yt3-G9. When the LIS-MV were added to the opposite type cells, no agglutination occurred but swimming was inhibited within a minute. Selfing pairs were induced in about 50 min if the inhibition of swimming was not disturbed. Neither inhibition of swimming nor induction of selfing pairs was observed when the vesicles were added to cells of the same mating type (Fig. 1). Figure 2 shows the mating-type-specific decrease in swimming velocity upon addition of the LIS-MV. Addition of about 50 μg/ml (protein concentration) LIS-MV decreased swimming velocity of opposite type cells to 50% of the normal velocity in a minute and to 15% in 10 min. The inhibition of swimming was reversible; cells once inhibited returned to the normal swimming speed when they were washed free from the LIS-MV (arrows in Fig. 2B).

Swimming velocity of selfing pairs induced by the LIS-MV was not affected by subsequent addition of the LIS-MV. Selfing pairs were induced by the LIS-MV (50 μg/ml) from cells of opposite mating type. About 7 hours after the addition of the vesicles, induced selfing pairs were washed with a solution containing 1 m*M* KCl, 1 m*M* $CaCl_2$, and 1 m*M* Tris-HCl, pH 7.1, and added with the LIS-MV (50 μg/ml) of opposite mating type. As seen in Table I, selfing pairs did not decrease their swimming velocity upon addition of the LIS-MV. This result is consistent with the fact that the cells, having formed conjugating pairs, lost their mating reactivity.

Observation of ciliary movement when the LIS-MV were added

When LIS-MV reacted cells were observed under the phase contrast microscope, immobilization of cilia on the ventral surface was seen. Some ventral cilia stuck together at their tips. This observation agreed well with the report by Hiwatashi (1961) that the mating reactivity of *P. caudatum* is restricted to the ventral side of the cell. However, the above observation does not mean that movement of the dorsal cilia is normal. Dorsal cilia did not show typical metachronal waves. An anterior shift of the beating direction and decrease in the beating angle of amplitude were observed. These results suggest that movement of dorsal cilia which lack mating agglutination ability, is also affected by interactions of mating-reactive surface membranes of ventral cilia with the LIS-MV.

Effect of LIS-MV on the swimming velocity of Triton-extracted models

To determine if only living cells show decrease in swimming velocity upon sexual recognition, the LIS-MV were applied to detergent-extracted models of the cells, in which the cell membrane is functionally disrupted.

Triton-extracted models of *Paramecium* were prepared by Naitoh and Kaneko's (1972) method with slight modifications. Cells were suspended in the extraction medium containing 0.005% (v/v) Triton X-100, 20 m*M* KCl, 10 m*M* EDTA, and 10 m*M* Tris-maleate buffer, adjusted to pH 7.0 with NaOH, at 0°C to 1°C. After treatment with the extraction medium for 30 min, the specimens were washed several times to remove the detergent, with a solution of 50 m*M* KCl and 10 m*M* Tris-maleate, pH 7.0. The models were reactivated by adding 4 m*M* ATP, 4 m*M* $MgCl_2$, and 3 m*M* K_3-EGTA to the washing solution 30 min after the washing.

When the models were treated with more than 100 μg/ml LIS-MV (the concentration being enough for the full inhibition of swimming in living cells), no change

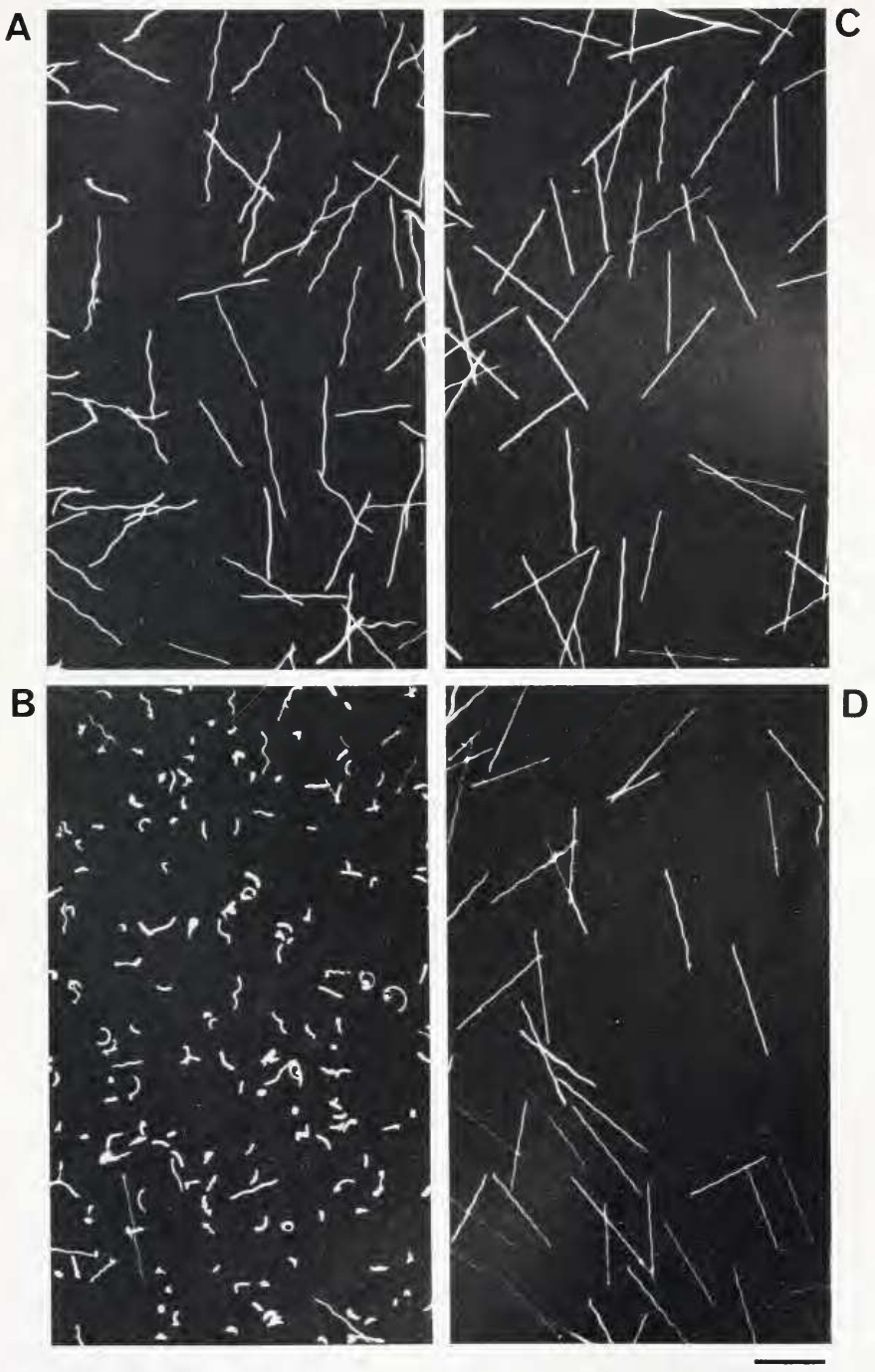

FIGURE 1. Tracks of swimming behavior of *P. caudatum* before and after the addition of the LIS-MV. The photographs were taken under dark-field illumination with two seconds exposure at 21°C. A, C; swimming tracks of cells before addition of the LIS-MV. B, D; swimming tracks of cells 10 min after addition of 40 μg/ml LIS-MV from stock Yt3-G9 (mating type VI). A, B; the opposite mating type cells, stock Kyky 1 (V). C, D; the same mating type cells, stock d-8b as the membrane vesicles added. Bar = 2 mm.

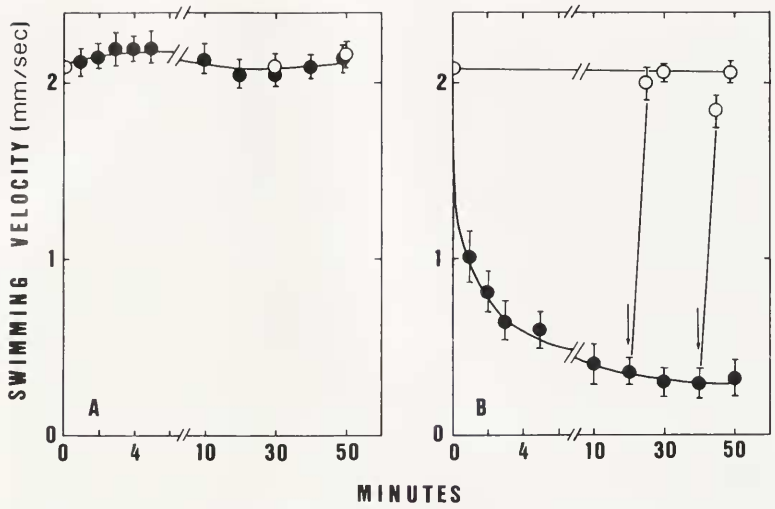

FIGURE 2. Mating type specific decrease in swimming velocity of *P. caudatum* by the LIS-MV. (A) Swimming velocity of the same mating type cells, stock Yt3-G9 as the vesicles added. (B) Swimming velocity of the opposite type cells (stock S7a). The abscissa represents the time in minutes after the addition of the LIS-MV. Ordinate, swimming velocity (mm/s) at 21°C. ●; With 50 μg/ml LIS-MV from stock Yt3-G9, mating type VI. ○; Without the vesicles. Cells were washed to remove the LIS-MV with a solution containing 1 m*M* KCl, 1 m*M* $CaCl_2$, and 1 m*M* Tris-HCl, pH 7.1, at the times indicated by arrows. Swimming velocity of the washed cells was measured after adaptation for one minute in the solution to settle the cells mechanically agitated by hand-operating centrifugation. Each swimming velocity was determined by about 30 measurements of different tracks.

in the swimming velocity of the models was seen (about 200 μm/s before and after the addition of the LIS-MV). The failure of inducing a decrease in swimming velocity of the models is not caused by failure of the attachment of the LIS-MV to the models. Mating-type specific agglutination of the models was induced by addition of more than 500 μg/ml of the LIS-MV. The results suggest that the inhibition of swimming is not induced by mere attachment of LIS-MV but that some membrane-physiological control of ciliary movement lacking in the Triton-extracted models is responsible for the inhibition of swimming.

TABLE I

*Effect of the LIS-MV on the swimming velocities of single cells and selfing pairs**

Cells of stock 27aG3	Swimming velocity (μm/s)[a]	
	−LIS-MV	+LIS-MV[b]
Single cells	1581 ± 185 (12)	461 ± 148 (12)
Selfing pairs	896 ± 233 (10)	862 ± 68 (9)

* Each result is the mean and 95% confidence limit. Numbers in parentheses indicate number of specimens.

[a] Swimming velocity was determined by measurements of about 10 swimming tracks of different cells by stroboscopic pulse exposures (six pulse exposure at the interval of 0.5 s each).

[b] The LIS-MV from stock Kyky 1 (mating type V).

Swimming behavior during chemical induction of conjugation

In *Paramecium,* various chemical agents under Ca-poor conditions induce selfing pairs among cells of a single mating type (Miyake, 1958, 1968). Selfing pairs appear in about 60 min in the induction medium without prior occurrence of agglutinative mating reaction. Cells show a marked change in their swimming behavior during the chemical treatment (Miyake, 1958; Tsukii and Hiwatashi, 1978; Cronkite, 1979). The change in the swimming behavior of the cells treated with the conjugation-inducing

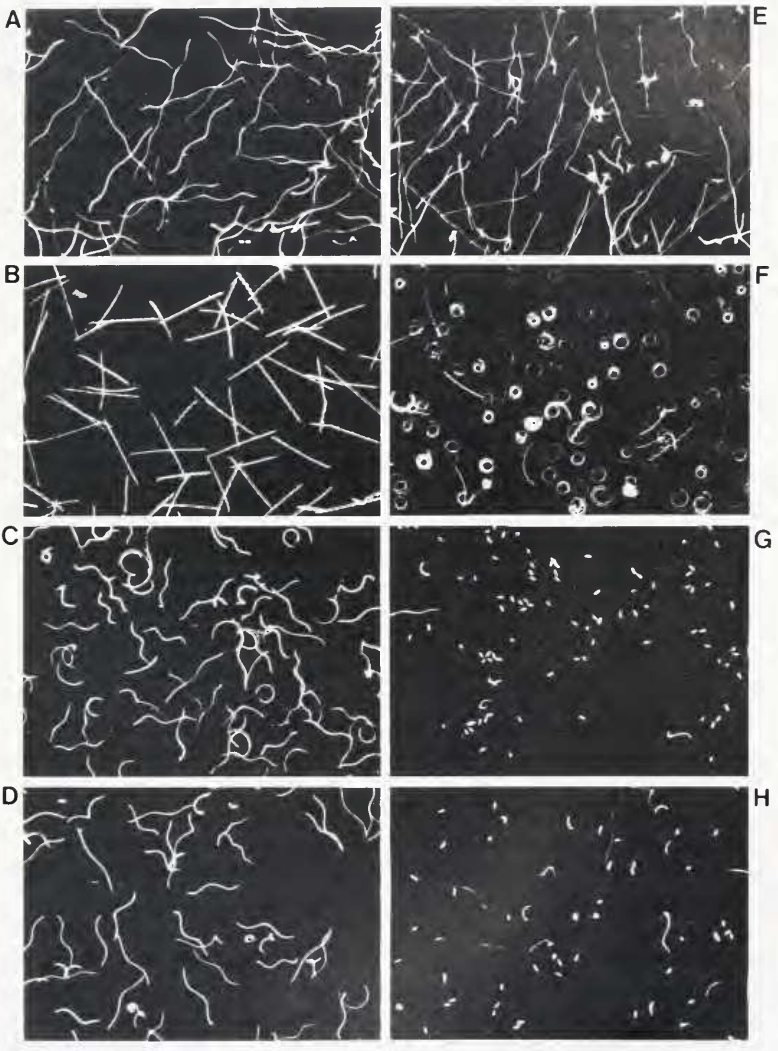

FIGURE 3. Photographs of swimming tracks of *P. caudatum* when introduced into a chemical induction medium. A–D; immature cells of stock 27aG3-S1. E–H; mating reactive mature cells of stock 27aG3-S1. A, E; adapting cells in a solution containing 1 m*M* KCl, 1 m*M* $CaCl_2$, and 1 m*M* Tris-HCl, pH 7.1 at 25°C. B, F; cells in the induction medium 30 s after the transfer. C, G; cells in the induction medium 10 min after the transfer. D, H; cells 40 min after the transfer. The photographs were taken under dark-field illumination with 2 s exposure. Bar = 2 mm.

chemicals consists of cell whirling accompanying a complete stop of forward swimming. For observation of the ciliary movement in the conjugation-inducing medium, we chose a very simple inducing medium containing only KCl and a trace of $CaCl_2$. Effects of these two cations on ciliary movement in *Paramecium* have been well analyzed electrophysiologically. Potassium is one of the best agents for the artificial induction of conjugation (Miyake, 1958).

For observation of swimming behavior of cells in conjugation-inducing chemicals, mating reactive cells were compared with non-reactive cells of the same genotype in the period of sexual immaturity. Thus, mating reactive cells of the complete homozygous strain 27aG3-S1 and immature cells of their selfing progeny were used. Figure 3 shows the change in swimming behavior of mating reactive and non-reactive cells upon treatment with conjugation-inducing chemicals. After being suspended in an adaptation medium containing 1 m*M* KCl, 0.03 m*M* $CaCl_2$, and 1 m*M* Tris-HCl,

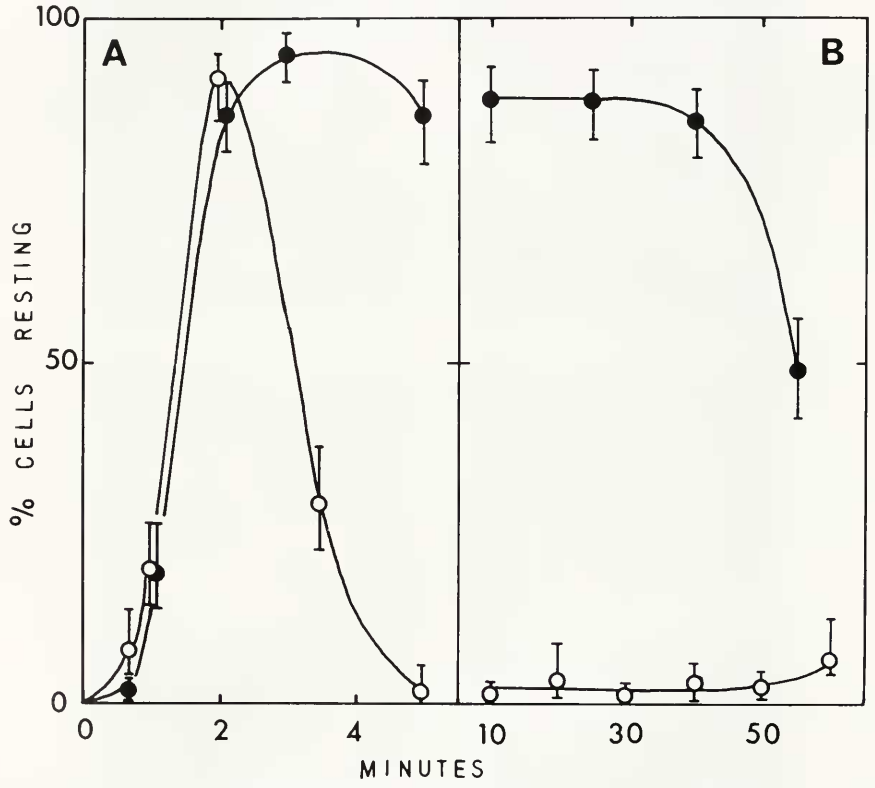

FIGURE 4. Mating reactivity-specific inhibition of swimming in the chemical induction medium. Abscissa; time after the transfer to the chemical induction medium (10 m*M* KCl, 0.03 m*M* $CaCl_2$, and 1 m*M* Tris-HCl, pH 7.1). Ordinate; percent of cells showing cessation of swimming in photographs taken with one second exposure. ○; Cells of stock 27aG3-S1 in sexually immature period. ●; Mating reactive mature cells of stock 27aG3-S1. (A) Time course within 5 min after the induction. Immediately after cells were transferred into the induction medium, one ml of the cell suspension was applied to a glass plate to take photographs at each time. (B) Time course from 10 to 60 min after the induction. One ml of sample cell suspension was applied onto glass plates at each time from the same batch. Photographs were taken one minute after the application of cells to the plates in order to settle the transferred cells from possible mechanical agitation.

pH 7.1, for 10 min, cells were transferred into the conjugation-inducing medium containing 10 m*M* KCl, 0.03 m*M* $CaCl_2$, and 1 m*M* Tris-HCl, pH 7.1 at 25°C.

Immature cells having no mating reactivity showed continued backward swimming for about 40 s. This appears as fine wavy tracks in the photograph (Fig. 3B). In contrast, mating reactive mature cells ceased backward swimming about 20 s after the transfer and showed a whirling motion (Fig. 3F). Another marked difference in swimming behavior between immature and mature cells occurred several minutes after transfer into the induction medium. More than 90% of reactive cells stopped swimming and continued to be in this state until the appearance of selfing pairs (Fig. 3G, H). Neither cessation of swimming nor formation of pairs was induced in immature cells (Fig. 3C, D). Figure 4 shows the percentage of non-motile cells during the chemical induction of conjugation. More than 90% of mating reactive cells showed cessation of swimming within 2 min and remained in this condition for more than 40 min, until the appearance of conjugating pairs. Conjugating pairs swim normally in the conjugation-inducing medium, whereas immature cells did not stop swimming, though their swimming behavior changed considerably in the medium (Fig. 3C). The increase in the number of resting cells in the figure (Fig. 4A) does not indicate the cessation of swimming in immature cells. It indicates whirling cells because cells at the end of backward swimming show small whirling circles. The tracks of such cells cannot be distinguished from those not swimming. Thus, dots in photographs include whirling cells which were scored as resting cells. The immature cells, however, regained their forward swimming shortly after such whirling. When the mating-reactive cells were washed free from the inducing medium with a solution containing 1 m*M* KCl, 1 m*M* $CaCl_2$, and 1 m*M* Tris-HCl, pH 7.1, and resuspended in the same solution, they recovered their forward movement immediately and returned to normal swimming behavior.

Calcium ions inhibit chemical induction of conjugation (Miyake, 1958; Hiwatashi, 1959). When the concentration of $CaCl_2$ was increased ten times that of the normal

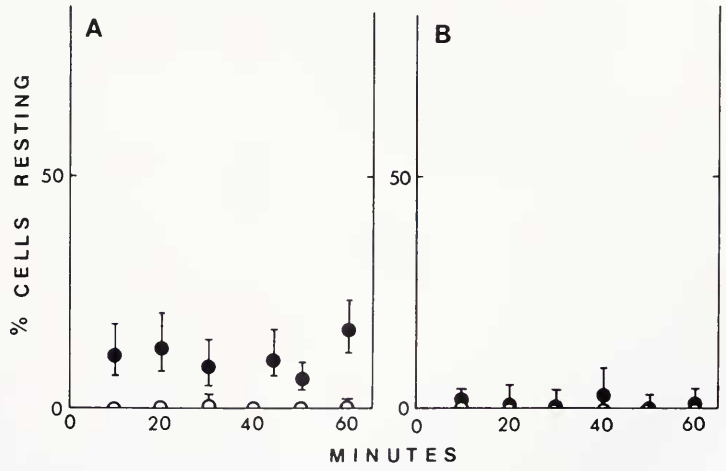

FIGURE 5. Inhibition of the cessation of swimming by Ca ions. (A) Cells in the solution containing 10 m*M* KCl, 0.3 m*M* $CaCl_2$, and 1 m*M* Tris-HCl, pH 7.1. (B) Cells in the solution containing 10 m*M* KCl, 3 m*M* $CaCl_2$, and 1 m*M* Tris-HCl, pH 7.1. Abscissa; time after the transfer to the solution, ordinate; percent of resting cells indicated as in Figure 4. ○; Immature cells of stock 27aG3-S1, ●; Mating reactive mature cells of the same stock.

induction medium, the number of conjugating pairs induced decreased to less than 10%. In this condition a noticeable decrease in the ratio of the resting cells was seen (Fig. 5A). Neither cessation of swimming nor pair formation was observed when the concentration of $CaCl_2$ was 100 times higher than the induction medium (Fig. 5B). These results clearly show a positive correlation between the cessation of swimming and the induction of conjugating pairs.

Observation of ciliary movement during chemical induction of conjugation

When mating reactive cells whose swimming was stopped in the induction medium were observed under a phase contrast microscope, perturbation of ciliary metachronal waves was seen not only in the ventral cilia but also over the dorsal cilia. Moreover, adhesion of some ventral cilia of the same cell at their tips were observed. This is almost the same change observed in cells treated with the LIS-MV.

Discussion

The inhibition of swimming induced by sexual recognition on the ciliary surface in *Paramecium* opens an interesting problem on regulatory mechanisms of ciliary movement. The inhibition appears to couple tightly with the trigger reaction in the conjugation process since conjugating pairs cannot be induced when the inhibition of swimming by the LIS-MV was disturbed by washing cells (Fig. 2B) or when cessation of swimming in the conjugation-inducing medium was inhibited by adding Ca ions (Fig. 5).

Machemer (1974) reported a positive correlation between membrane depolarization and change in ciliary beat frequency or shift of the beat direction. Preliminary results obtained by direct measurement of membrane potential, however, suggest that the ciliary inactivation induced by mating recognition with the LIS-MV is not controlled by the membrane potential change (Kitamura *et al.*, 1980). Furthermore, it takes about 5 min for the cells reacted with the LIS-MV to show complete inhibition of swimming (Fig. 2). If any electrical change in cell surface membrane was responsible for the inhibition of ciliary movement by sexual recognition, the response should be much faster than that obtained. We recently detected an increase in adhesiveness of the ventral cilia to polystyrene surfaces during the mating reaction or the chemical induction of conjugation (Kitamura, unpub. result). This change in the adhesiveness of cells proved to result from an increase in hydrophobicity of surfaces of the ventral cilia (Kitamura, 1982). Thus, ciliary membranes with mating reactivity become more sticky in the conjugation-inducing medium or by the attachment to the LIS-MV and hence agglutinate each other. This change could cause the inactivation of ciliary movement and perturbation of ciliary coordination observed in this study. The result with Triton-extracted models where swimming velocity did not decrease following LIS-MV exposure apparently conflicts with this hypothesis. As described in the results, however, the swimming velocity of the model made from mating reactive cells was about 200 μm/s, while that of the model from non-reactive cells (sexually immature) was about 260 μm/s. This suggests that some inactivation of ciliary movement in the mating reactive models had already occurred in the reactivation medium before adding the LIS-MV, because the reactivation medium itself has a strong conjugation-inducing activity as seen in its ionic condition, high in K^+ concentration and low in Ca^{2+} concentration (50 m*M* KCl and 3 m*M* EGTA). We do not know whether adhesion of ventral cilia occurs in this condition. But, if the adhesion had already occurred, addition of the LIS-MV would not decrease the swimming velocity further.

The observation that the models from mating reactive cells tend to swim in a twisting manner upon reactivation but those from cells without mating reactivity do not, agrees well with this explanation.

An important role of Ca^{2+} has been suggested in the activation-initiating mechanism of conjugation in *Paramecium* (Cronkite, 1979; Hiwatashi, 1981) such as that of fertilization in metazoan eggs (Mazia, 1937; Monroy, 1947; Epel, 1980). If no change in membrane potential was observed after application of the LIS-MV as mentioned before, rapid influx of Ca^{2+} into the cell might not occur during the mating reaction. The above conclusion agrees well with the facts that formation of conjugating pair by the mating reaction is not inhibited by the Ca-channel blocker $LaCl_3$ (Cronkite, 1977) and natural conjugation occurs in all known mutants with defective Ca-channels (Cronkite, 1974; Takahashi, 1979). Therefore, a membrane potential change such as the fertilization potential which functions as a fast block to polyspermy in echinoderms (Jaffe, 1976; Miyazaki and Hirai, 1979), echiuriens (Gould-Somero *et al,* 1979), and amphibians (Cross and Elinson, 1980; Grey and Schertel, 1978) appears unnecessary for the activation of conjugation in *Paramecium.*

The mating-type-specific ciliary inactivation or decrease in swimming velocity reported here can be used as a good method for assaying the mating substances since it is a very simple and clear indicator of activation of conjugation in *Paramecium.*

LITERATURE CITED

Cronkite, D. L. 1974. Genetics of chemical induction of conjugation in *Paramecium aurelia. Genetics* **76:** 703–714.

Cronkite, D. L. 1977. An analysis of the mechanism of activation of rapid macronuclear migration, a very early event in conjugation of *Paramecium caudatum. Proc. Int. Congr. Protozool.* 5th, New York. pp. 284 (abst.).

Cronkite, D. L. 1979. The genetics of swimming and mating behavior in *Paramecium.* Pp. 221–273 in *Biochemistry and Physiology of Protozoa,* vol. 2, M. Levandowsky and S. H. Hutner, eds. Academic Press, New York.

Cross, N. L., and R. P. Elinson. 1980. A fast block to polyspermy in frogs mediated by change in the membrane potential. *Dev. Biol.* **75:** 187–198.

Dryl, S. 1959. Antigenic transformation in *Paramecium aurelia* after homologous antiserum treatment during autogamy and conjugation. *J. Protozool.* **6:** suppl. 25.

Epel, D. 1980. Experimental analysis of the role of intracellular calcium in the activation of the sea urchin egg at fertilization. Pp. 169–185 in *The Cell Surface: Mediator of Developmental Processes,* S. Subtelny and N. K. Wessells, eds. Academic Press, New York.

Gould-Somero, M., L. A. Jaffe, and L. Holland. 1979. Electrically-mediated fast polyspermy block in eggs of the marine worm, *Urechis caupo. J. Cell Biol.* **82:** 426–440.

Grey, R. D., and E. R. Schertel. 1978. Ionic induction of polyspermy in *Xenopus:* Evidence for a fast block. *J. Cell Biol.* **79:** 164a (abst.).

Hiwatashi, K. 1959. Induction of conjugation by ethylenediaminetetraacetic acid (EDTA) in *Paramecium caudatum. Sci. Rep. Tohoku Univ. Ser. IV* **25:** 81–90.

Hiwatashi, K. 1961. Locality of mating reactivity on the surface of *Paramecium caudatum. Sci. Rep. Tohoku Univ. Ser. IV* **27:** 93–99.

Hiwatashi, K. 1968. Determination and inheritance of mating type in *Paramecium caudatum. Genetics* **58:** 373–386.

Hiwatashi, K. 1981. Sexual interactions of the cell surface in *Paramecium.* Pp. 351–378 in *Sexual Interactions in Eukaryotic Microbes,* D. H. O'Day and P. A. Horgen, eds. Academic Press, New York.

Jaffe, L. A. 1976. Fast block to polyspermy in sea urchin eggs is electrically mediated. *Nature* **261:** 8–71.

Kitamura, A. 1982. Attachment of *Paramecium* to polystyrene surfaces: A model system for the analysis of sexual cell recognition and nuclear activation. *J. Cell Sci.* **58:** 185–199.

Kitamura, A., and K. Hiwatashi. 1978. Are sugar residues involved in the specific cell recognition of mating in *Paramecium*? *J. Exp. Zool.* **203:** 99–108.

Kitamura, A., and K. Hiwatashi. 1980. Reconstitution of mating active membrane vesicles in *Paramecium. Exp. Cell Res.* **125:** 486–489.

Kitamura, A., H. Onimaru, Y. Naitoh, and K. Hiwatashi. 1980. Ciliary inactivation following sexual cell recognition in *Paramecium. Dev. Growth Differ.* **22:** 722 (abst.).

MACHEMER, H. 1974. Frequency and directional responses of cilia to membrane potential changes in *Paramecium. J. Comp. Physiol.* **92:** 293–316.

MAZIA, D. 1937. The release of calcium in arbacia eggs on fertilization. *J. Cell. Comp. Physiol.* **10:** 291–304.

MIYAKE, A. 1958. Induction of conjugation by chemical agents in *Paramecium caudatum. J. Inst. Polytech. Osaka City Univ. Ser. D* **9:** 251–296.

MIYAKE, A. 1968. Induction of conjugation by chemical agents in *Paramecium. J. Exp. Zool.* **167:** 359–380.

MIYAZAKI, S., AND S. HIRAI. 1979. Fast polyspermy block and activation potential: Correlated changes during oocyte maturation of a starfish. *Dev. Biol.* **70:** 327–340.

MONROY, A. 1947. Further observation on the fine surface of the cortical layer of unfertilized and fertilized sea urchin eggs. *J. Comp. Physiol.* **30:** 105–109.

NAITOH, Y., AND H. KANEKO. 1972. Reactivated Triton-extracted models of *Paramecium:* Modification of ciliary movement by calcium ions. *Science* **176:** 523–524.

SONNEBORN, T. M. 1937. Sex, sex inheritance and sex determination in *Paramecium aurelia. Proc. Natl. Acad. Sci. U.S.A.* **23:** 378–385.

TAKAHASHI, M. 1979. Behavioral mutants in *Paramecium caudatum. Genetics* **91:** 393–408.

TSUKII, Y., AND K. HIWATASHI. 1978. Inhibition of early events of sexual processes in *Paramecium* by concanavalin A. *J. Exp. Zool.* **205:** 439–446.

TSUKII, Y., AND K. HIWATASHI. 1979. Artificial induction of autogamy in *Paramecium caudatum. Genet. Res.* **34:** 163–172.